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## **Schlieren laser Doppler flowmeter for the human optical nerve head with the flicker stimuli**

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# Schlieren laser Doppler flowmeter for the human optical nerve head with the flicker stimuli

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**Abstract.** We describe a device to measure blood perfusion for the human optic nerve head (ONH) based on laser Doppler flowmetry (LDF) with a flicker stimuli of the fovea region. This device is self-aligned for LDF measurements and includes near-infrared pupil observation, green illumination, and observation of the ONH. The optical system of the flowmeter is based on a Schlieren arrangement which collects only photons that encounter multiple scattering and are back-scattered out of the illumination point. LDF measurements are based on heterodyne detection of Doppler shifted back-scattered light. We also describe an automated analysis of the LDF signals which rejects artifacts and false signals such as blinks. By using a Doppler simulator consisting of a lens and a rotating diffusing wheel, we demonstrate that velocity and flow vary linearly with the speed of the wheel. A cohort of 12 healthy subjects demonstrated that flicker stimulation induces an increase of 17.8% of blood flow in the ONH. © 2013 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.18.12.127001]

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## 1 Introduction

Laser Doppler flowmetry (LDF) in the optic nerve head (ONH) was first developed by Riva et al. in 1982<sup>1</sup> and adapted in 2009.<sup>2</sup> Modified Topcon and Kowa fundus cameras were used for clinical studies of ONH blood flow changes induced by different techniques such as perfusion pressure changes after intraocular pressure increase,<sup>3,4</sup> blood pressure changes,<sup>5</sup> gas breathing,<sup>6</sup> or flicker light.<sup>4,7</sup> Such instruments provide a very little information about the prelaminar and deeper regions of the ONH.<sup>8</sup> However, an increase of the distance between the illumination and collection position<sup>9</sup> would provide information about deeper regions.

The optical set-up is based on a pseudoconfocal arrangement (Schlieren) in which illumination pupil (annulus) and detection pupil (disk within the annulus) are separated, where the observation of the illumination point is blocked by an annulus field stop (Fig. 1). Such an optical system is auto-aligned and only multiple back-scattered light can be detected. The size of the blocking disk of the field stop determines the distance between the illumination and the collection light. This way of collecting light partially eliminates the problem of confocal laser Doppler measurements,<sup>10</sup> which are very sensitive to saccades and movements because of speckles and collect mainly the superficial blood flow information. In this way, the optical arrangement is closer to the technique used for laser Doppler skin measurements, where the illumination and detection are widely separated. In such a situation, the depth of measurement and the probed volume increased with the distance between the source and the detection.<sup>11</sup>

## 2 Description of the Device

### 2.1 Optical System

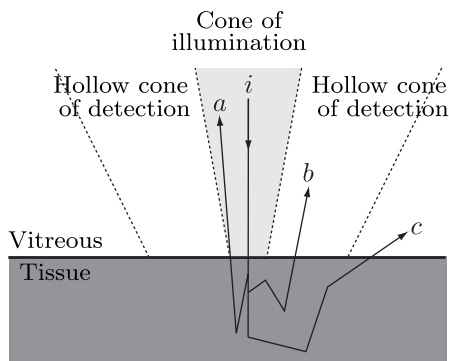
The device consists of five optical systems (Fig. 2): green illumination of the ONH, observation of the ONH, Schlieren LDF unit, projection of a fixation point coupled with the projection of a two wavelengths flicker illumination of the fovea and its surroundings and dedicated illumination, and observation of the eye pupil, which is not shown in Fig. 2. Observation of the ONH, fixation and flicker for the fovea and Schlieren LDF are the identical optical systems (lenses  $L_{o1} = L_{f1}$ ,  $L_{o2} = L_{f2} = L_{s2}$ ) with a magnification of 2.27 from the fundus to the last image. Conjugate pupils are labeled as  $P_x$  and conjugate images as  $I_x$ .

**Illumination of the ONH:** A two lens system images a diffusing light source (505 nm, 20 nm full width at half maximum) onto the ONH with a field limitation of  $\pm 3$  deg. The light pathway is separated from the other optical systems.

**Observation of the ONH:** Lenses  $L_{o1}$  and  $L_{o2}$  image the fundus  $I$  onto a charge coupled device camera (B&W camera, 659×494, imaging source) placed at  $I_1$ . An example of a fundus image is given in Fig. 3. In the eye pupil, light footprints of the illumination and observation are separated by 3 mm in order to avoid back-reflection from the illumination into the observation path.

**Schlieren LDF unit:** Probing light, located in the plane  $I_4$ , is delivered by a laser diode at 785 nm with a power of 90  $\mu$ W at the eye pupil, which conforms to the American National Standard Institutes for laser safety.<sup>12</sup> The beam-splitter  $BS_o$  reflects near-infrared (NIR) and an absorption filter  $F$  ensures that only the probing light is detected at  $I_3$  by an avalanche photodiode (C5460-1, Hamamatsu, Japan). An aperture stop of 2.4-mm diameter at  $P_3$  limits the probing laser beam and

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**Fig. 1** An incident photon  $i$  within the cone of illumination can encounter three kinds of back-scattering:  $a$ , within the cone of illumination which is blocked later in the optical system,  $b$ , which encounters multiple scattering that comes back within the hollow cone of detection, and  $c$ , which will not be collected by the optical system.

an annulus aperture (from 2.4 to 8 mm in diameter) at  $P_2$  limits the detected light. This avoids reflected laser light at the eye pupil ( $P$ ). Pupils  $P_2$  and  $P_3$  are placed on the faces of the polarizing beam-splitter PBS. The Schlieren arrangement consists of an annulus field stop (from 45 to 300  $\mu\text{m}$  in diameter) in the detection plane  $I_3$  and of a point source at the probing light source plane  $I_4$ .

Fixation point and flicker illumination of the fovea: Optical axis of the fixation and the observation build an angle of 15 deg. The fixation point in plane  $I_2$ , which can be moved perpendicularly to the optical axis, consists of a 670-nm laser diode of 1.2  $\mu\text{W}$  at the eye pupil  $P$ . By asking the subject to look at this point located in the foveola, the probing beam can be placed on the desired location on the ONH for an LDF recording. Along the optical path to the fovea, a dichroic beam-splitter  $BS_f$  placed at a conjugate pupil plane reflects light coming from two LEDs (470 and 525 nm) modulated at 10 Hz,

which illuminates the fovea and its surroundings ( $\pm 6$  deg) with an intensity of 4  $\mu\text{W}$ . When one of these LEDs is turned on, the intensity of the illumination of the ONH is also modulated in phase and in frequency with the flicker illumination.

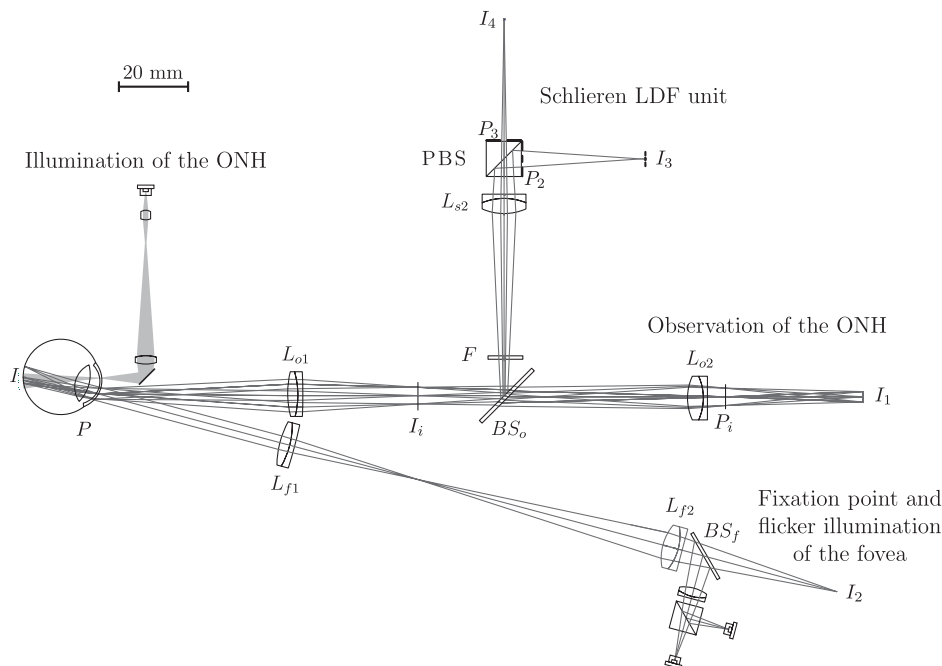
The entrance position of the probing beam into the eye, which defines the angle of the incidence of the probing beam onto the ONH and thus the Doppler frequency shift, should remain the same from one measurement to the other. To ensure this, a second camera points to the eye pupil, which is illuminated by a dedicated NIR light. Thus the entrance position of the probing beam within the eye pupil can be checked and if necessary the head of the subject can be adjusted.

## 2.2 Signal Analysis

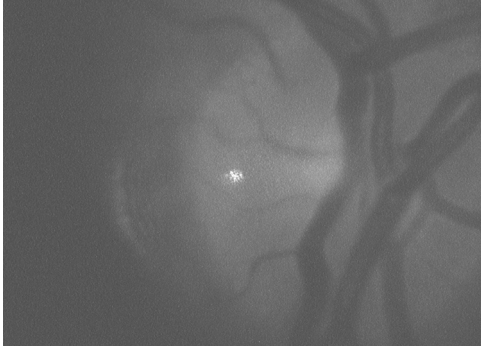
Bonner and Nossal<sup>13</sup> proposed a theoretical model, which was primarily intended for scattering tissues such as skin, but not the eye fundus. Based on this model, a fast Fourier transform computer implementation for measuring blood flow in the ONH was used.<sup>14</sup> Using the same analysis technique, a new code was written in LabView and first tested with a MATLAB procedure. Substantial automatic analysis based on noise level, signal amplitude, and power spectrum level were implemented and explained below. In this theory, a probabilistic model is postulated for the interaction between photons and red blood cells. The relation between power spectrum of Doppler-shifted light  $PS(f)$  and laser Doppler perfusion parameters volume, Vol, in arbitrary units and velocity, Vel, in Hertz can be established quantitatively as

$$\int_0^{\infty} f \cdot PS(f) df \sim \text{Vel}, \quad (1)$$

$$\int_0^{\infty} PS(f) df \sim \text{Vol}. \quad (2)$$



**Fig. 2** Optical system with the illumination (ONH and fovea for the flicker light), observation and LDF system. Indices  $o$ ,  $f$ , and  $s$  stand, respectively, for the paths of observation, fixation/flicker and detection, and  $i$  for intermediate.  $I$  and  $P$  stand for images and pupils conjugate planes, respectively. The illumination and observation of the eye pupil are not shown here.



**Fig. 3** Example of an image of the fundus is taken by the instrument. The probing beam can be seen in the middle of the image.

The power spectrum  $P(f)$  of the detector output current (or, accordingly, the one of the digital signal  $x[n]$ ) is not equal to or proportional to the Doppler-shifted light spectrum  $PS(f)$ , but is also influenced by a nonzero DC and by a shot-noise.<sup>13,15</sup> In order to estimate Vel and Vol, the following quantities are computed:

$$\frac{1}{\bar{x}^2} \int_{f_{\min}}^{f_{\max}} f \cdot [P(f) - \hat{E}] df, \quad (3)$$

$$\frac{1}{\bar{x}^2} \int_{f_{\min}}^{f_{\max}} (P(f) - \hat{E}) df, \quad (4)$$

where  $\hat{E}$  is an estimate of the shot-noise of the power spectral density, assumed to be a constant (white noise). The following method is applied: The discrete power spectrum density (PSD)  $P(f_k)$  is estimated by the Welch method, dividing the input signal  $x[n]$ , sampled at  $f_s = 120$  kHz, into frames of length  $N = 8192$ . For each frame, the power spectrum  $P_i(f_k)$  is computed. Due to the movements of the eye, microsaccades and closing of eyelids,  $x[n]$  is not fully stationary. As a consequence, averaging  $P_i(f_k)$  to get the best estimate of  $P(f_k)$  is not performed over all frames, but, instead, specifically “atypical” frames are discarded. The shot-noise  $\hat{E}$  is estimated as the mean value of  $P(f_k)$  around  $f_{\max}$ . Vel and Vol are computed from the shot-noise free PSD  $P(f_k) - \hat{E}$  as specified above. To avoid negative values, the difference is replaced by zero when the values would be negative.  $f_{\min}$  is chosen as 30 Hz to discard DC, lower frequencies induced by artifact movement,<sup>16</sup> and the influence of the window used for FFT. Finally, relative BF = Vel  $\times$  Vol in arbitrary units gives a value related to the number of red blood cells moving in the tissue.

As mentioned above, the software performs and analyzes the Fourier transform of the photocurrent  $i(t)$  with a bandwidth of 120 kHz at a rate of 14.7 acquisitions per second. It automatically removes data when a sudden change in the blood volume occurs, which is mainly due to microsaccades, if power spectrums are too low, i.e., close to the noise level and if the DC of the data is outside a range of  $\pm 10\%$  of the most probable value of the whole recording.

Due to the fact that back-scattering is a function of the optical properties of the eye (cornea, lens, vitreous, ONH tissue) and the angle of the incident and collecting beams, only changes in LDF perfusion parameters Vol, Vel, and BF are relevant. The absolute values cannot be compared between subjects.

### 3 Experiments

The study was conducted in accordance with the Declaration of Helsinki for a research involving human subjects and with Good

Clinical Practice Guidelines. Informed consent was obtained from the subjects after the explanation of the study. The study protocol was approved by the Local Institutional Review Board (IRB #6705) and was registered on ClinicalTrials.gov (NCT00874913).

#### 3.1 Linearity

A simulator, consisting of a single lens (20-mm focal length) and a rotating Teflon<sup>®</sup> wheel, is used to produce Doppler shifts. All teflon scattering particles move approximately with the same velocity. Frequency shifts of light increase with the multiple scattering and in turn decrease as it decreases. With the chosen geometry, the induced power spectrum is close, but not identical, to that obtained from the human eye, i.e., Vol and Vel have similar values. Because the amount of back-scattered light is independent of the rotating speed, Vol should be constant, Vel is expected to be linear with the rotating speed and consequently BF, too. The speed of the surface of the wheel, which scatters light, is proportional to the rotating frequency  $f$ .

#### 3.2 Sensitivity

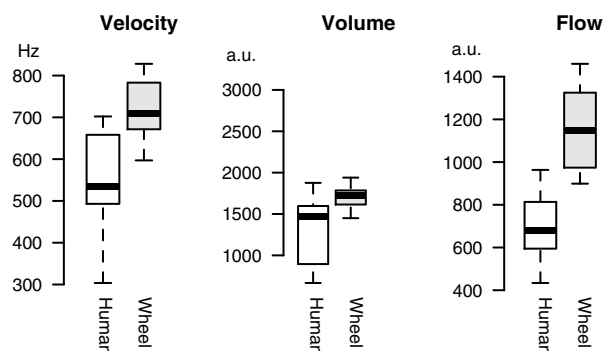
The minimum statistically significant change in an LDF parameter that can be detected with the device for a given experiment with  $N$  subjects is defined as

$$S = \frac{SD}{\bar{x}} \times k \times \sqrt{N}, \quad (5)$$

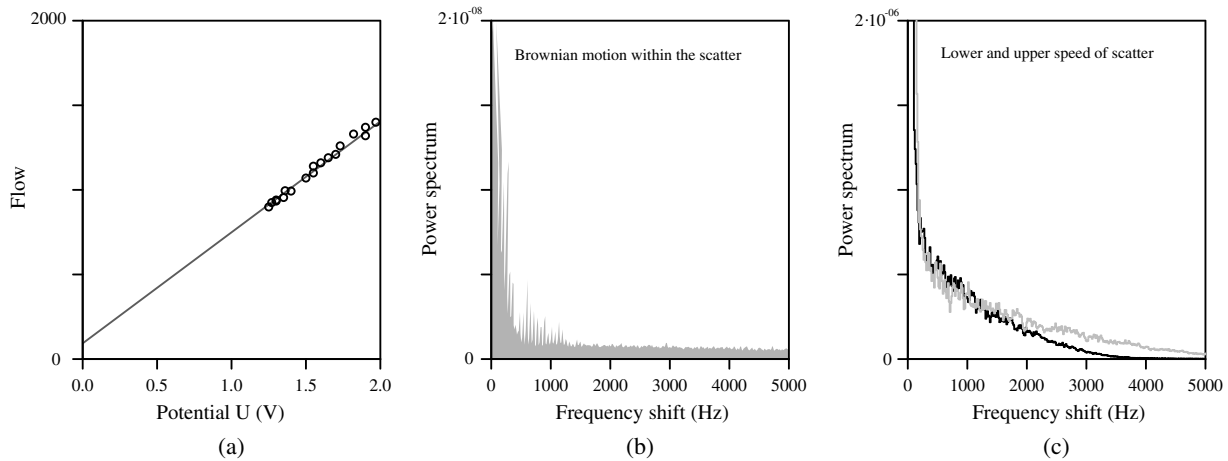
where  $k$  is the two-tailed value of the  $t$ -distribution at 0.05 significance level for  $N - 1$ ,  $\bar{x}$  is the mean value of all measurements, and SD is the standard deviation of the difference between two subsequent recordings of each subject.<sup>17</sup>

#### 3.3 Response to Flicker

The pupil of the fixator eye of each subject was dilated with one drop of tropicamide 0.5%. After a rest of 20 min, the subject was asked to look at the fixation point and the probing laser was directed to the temporal superior field of the ONH in the tissue, where there were no vessels (Fig. 3). A continuous recording of 80 s was started. After 20 s, the 10 Hz flicker light was applied for 40 s. Statistical changes of BF were analyzed with a programming language  $R$  ([www.r-project.org](http://www.r-project.org)) and SPSS statistics.



**Fig. 4** Blood flow parameters for the cohort of 12 subjects at the baseline and the 17 measurements done at different rotating speeds give similar results. The thick line within the box is the median, the box height represents the first quartile of the data and the upper and lower lines are 1.5 interquartile distance.



**Fig. 5** (a) Calculated flow as a function of the speed of the wheel. (b) Power spectrum of the wheel at rest, which is about 100× lower when the wheel is rotating. (c) Power spectrum at the lowest (black) and the highest (gray) speeds. A power spectrum obtained from the human eye is very similar.

Data were analyzed using the mixed models procedure. (LDF parameters were defined as repeated measures, flicker was defined as a fixed effect during the 20- to 60-s period, and subject was defined as random effect.) The entire flicker period was selected to quantify the BF, Vel, and Vol changes.

## 4 Results

### 4.1 Linearity

The rotating wheel does not simulate a perfused tissue, but produces a power spectrum similar to those of ONH (Fig. 4).

With  $U$  the applied voltage on the motor, we found that the rotating frequency  $f$  of the motor is a linear function of  $U$ :  $f = (0.250 \pm 0.001 \text{ Hz/V}) U - (0.036 \pm 0.008 \text{ Hz})$ .

Therefore, in the following, the flow parameters are expressed as a function of the voltage  $U$ .

As expected, Vel is highly linearly dependent of  $U$  ( $\text{Vel} = 233 \cdot U + 341$ ;  $R^2 = 0.87$ ;  $p < 0.0001$ ). Vol, which should be independent, exhibits a slight increase over the whole span of the voltage used for that experiment ( $\text{Vol} = 408 \cdot U + 1032$ ;  $R^2 = 0.66$ ;  $p < 0.0001$ ). Finally, BF is also linearly dependent of the voltage [ $\text{BF} = 656 \cdot U + 93$ ;  $R^2 = 0.98$ ;  $p < 0.0001$ , see Fig. 5(a)].

### 4.2 Sensitivity

Within the flicker experiment, we considered the baseline as two recordings of 10 s. For the 12 subjects, we found a sensitivity at  $p = 0.05$  of 5.3%, 12.4%, and 7.6% for Vel, Vol, and BF, respectively.

### 4.3 Response to Flicker

Twelve healthy caucasian subjects were included [ $30.0 \pm 2.3$  (SEM) years old,  $-0.8 \pm 0.3$  diopters, nine men and three women]. During flicker stimulation, the increase of blow flow ( $17.8 \pm 12.9\%$ ,  $p < 0.001$ ) was concomitant with the rise of velocity ( $+17.4 \pm 12.7\%$ ,  $p < 0.001$ ). Volume did not significantly change ( $p = 0.16$ ).

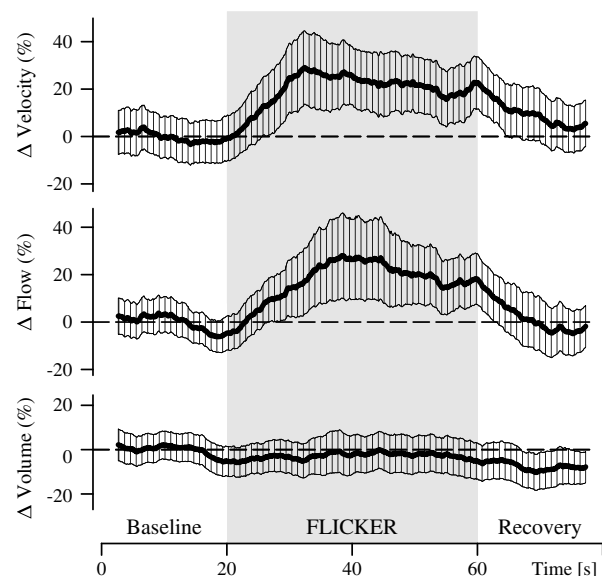
Linear fits between Vol or Vel and DC give coefficients of determination  $R^2$  smaller than 0.19. A larger value ( $R^2 = 0.42$ ) is found between Vel and Vol. A Pearson's correlation gives slightly higher values (0.27 and 0.59).

## 5 Discussion

In the present study, a flowmeter was evaluated. It is based on a Schlieren optical arrangement which collects only photons that encounter multiple scattering and is collected outside the illumination point. Linearity and sensitivity of the instrument were evaluated using a Doppler simulator and an experiment already published<sup>18</sup> was repeated.

With the Doppler simulator, Vel is linearly correlated with the speed of the wheel. The y-intercept of the linear fit represents Brownian motion [see Fig. 5(b)]. Vol is expected to be constant, but our measurements exhibit a slight increase. This is probably due to the fact that signals with frequencies higher than only 5 kHz are used to evaluate the shot-noise  $\bar{E}$  and thus influence the calculation of Vol [see Eq. (4)], but the change of Vol within the range of this experiment remains within  $\pm 3\%$ .

The value found for the sensitivity is in the range of those presented in the earlier papers for others experiments.<sup>10,17,19</sup>



**Fig. 6** Moving average ( $\pm 2.5$  s) of 12 recordings. The left part corresponds to the baseline, the central gray part to the flicker excitation, and the right part to the recovery. Confidence intervals are the standard error of the mean (SEM).

As a comparison, the coefficients of variation of perfusion parameters (Vel, Vol, BF) between a baseline and a second measurement 12 h later in 16 subjects using scanning LDF—instead of our instrument—were known and were  $15.5 \pm 5.4\%$ ,  $10.0 \pm 3.4\%$ , and  $15.6 \pm 5.2\%$ , respectively.<sup>20</sup>

As expected during the flicker experiments, the Vel time course and amplitude (+17%) are consistent with the previously published data. The Vel and BF time course increase rapidly to a plateau during flicker illumination and then decrease gradually. This pattern is consistent with the flow measurement of the ONH using other LDF techniques described by Riva et al.<sup>18,21</sup>

To explain the increase of Vol<sup>18</sup> induced by neurovascular coupling during flicker stimulation, the hypothesis of capillary vasodilatation was the most probable underlying mechanism, especially since some studies suggest that nitric oxide is implicated in the activity-induced BF response.<sup>21</sup> Nevertheless, the intrinsic optical properties of ONH influence the light intensity reflected from the active tissue. A change in the scattering properties would change intensity of the reflected light. Riva has clearly shown that there is a significant relationship between DC changes proportional to the reflected light intensity changes, and BF changes during flicker experiments.<sup>21</sup> A decrease of reflectance during flicker at isobestic wavelength and nonisobestic wavelength was shown. This was interpreted as a change in the blood volume. However, the decrease is higher for shorter wavelengths which suggests a change in the scattering properties and not only in the blood volume.<sup>22</sup> The exact link between reflectance change and capillary vasodilatation still remains to be explained. Both are involved in measured Vol time course response to flicker, but the part of each in the response is unknown. In this study, the absence of Vol changes could be explained by the different techniques used by the Schlieren LDF (multiple scattering, see Fig. 1) and that of Riva's LDF device (single scattering). These results suggest that reflectance changes of active ONH tissue during flicker might influence Vol measurements (Fig. 6).

Weak  $R^2$  confirms the independence of Vol and Vel toward DC, and suggests that Vol and Vel are poorly linked with DC and thus are independent.

## 6 Conclusion

The device presents a highly linear response to blood perfusion changes measured with a Doppler simulator. It exhibits a good sensitivity for the flicker experiment. The auto-adjustment of the probing beam with the detection is very convenient and location of measurement is easily targeted. Additionally, the Schlieren optical setup uses a larger spot size than the other LDF techniques and has proven to be robust and not affected by surrounding vessels. These preliminary data demonstrate that the instrument is reliable and can be used for further blood perfusion studies of the ONH.

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